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Cellular mechanism of hormone action in the kidney: Messenger function of calcium and cyclic AMP

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Considerable progress has been made in the past decade to define the messenger functions of Ca^{2+} and cyclic AMP in the actions of peptide and amine hormones [1–8]. It has become increasingly apparent that these two intracellular messengers serve interrelated rather than separate functions. To emphasize the interrelated nature of their actions and note the nearly universal association of the cyclic AMP messenger system with the calcium messenger system in the regulation of cellular responses, the concept of synarchic (syn—together; archon—ruler or herald) regulation was introduced [3]. The central theme of this concept is that in nearly all animal cells, Ca^{2+} and cyclic AMP serve together to regulate the cell response to this class of extracellular messengers. However, rather than functioning in a single stereotyped fashion, the interactions between these two systems display an amazing plasticity. As a consequence, the nature of their interactions vary from tissue to tissue. Within this variety of expression, five general patterns of Ca^{2+}-cyclic AMP interactions can be identified: coordinate, hierarchical, redundant, antagonistic, and sequential. In any given tissue, it is often possible to describe the major mode of control as conforming to one of these five patterns. However, when one does so, it is equally common to find that one or another of the other patterns provides a minor theme in the regulatory repertoire.

Within the past 3 to 4 years there has been considerable progress in defining the more intimate details by which events within these two messenger systems are interrelated, particularly those dealing with calcium. Much of this new knowledge has been gained from studies of hormone action in tissues other than the kidney. Yet, if one agrees with the authors that mammalian cells are more alike than unlike, then the knowledge gained from studies of angiotensin II action in liver, adrenal glomerulosa, and smooth muscle cells is directly relevant to the action of this hormone on proximal tubular function particularly since, in the other three tissues, the basic mechanism by which this hormone acts appears to be the same. Likewise, studies of the action of ACTH on adrenal glomerulosa cells may well be relevant to those of PTH on nephron segments. Thus, rather than review in detail the actions of angiotensin II, PTH, and vasopressin on the renal tubule as has been done quite recently by others [9–12], this review will summarize data obtained largely from studies in other tissues and then consider the actions of kidney hormones in the light of these data and the models of cell activation constructed from them.

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Behavior of the calcium messenger system during sustained cellular responses

Our major focus in this review will be a consideration of how the calcium messenger system functions to couple a hormonal stimulus to a sustained cellular response [8]. Four aspects of this type of process will be discussed: *(1)* the initial transducing events which occur in the plasma membrane when hormone interacts with its receptor, *(2)* the effect of the hormone on the calcium ion concentration in the cell cytosol, *(3)* the effect of the hormone on cellular calcium metabolism, and *(4)* the mechanisms by which gain control is achieved in the calcium messenger system. The latter will involve a consideration of how the cyclic AMP messenger system interacts with the calcium messenger system.

Initial membrane events

Nearly 30 years ago Hokin and Hokin [13] noted that the addition of carbachol to slices of acinar pancreas increased the incorporation of [^{32}P]-phosphate into phospholipids, and, in particular, into phosphatidylinositol. Twenty years of work led to the repeated demonstration that in cells in which Ca^{2+} seemed to serve a messenger function, the interaction of extracellular messenger with its receptor induced a turnover of phosphatidylinositides. These data led Michell [14] to propose that PI turnover and plasma membrane calcium gating were linked in some cause and effect fashion. Within the past few years improved methods for studying the metabolism of polyphosphatidylinositols has led to an understanding of the key role that these compounds play in the calcium messenger system [2, 15–17]. Although several aspects remain to be completely validated, present evidence support a model in which phosphatidylinositol 4,5-bisphosphate (PIP_2) is the critical plasma membrane component which undergoes phosphodiesteric cleavage when hormone interacts with receptor (Fig. 1) As a consequence, two products are generated; diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP_3). In addition, in some cells at least, hormone receptor interaction leads to an increase in the rate of synthesis of phosphatidylinositol (PI) and phosphatidylinositol 4-phosphate (PIP), the immediate precursors of PIP_2.

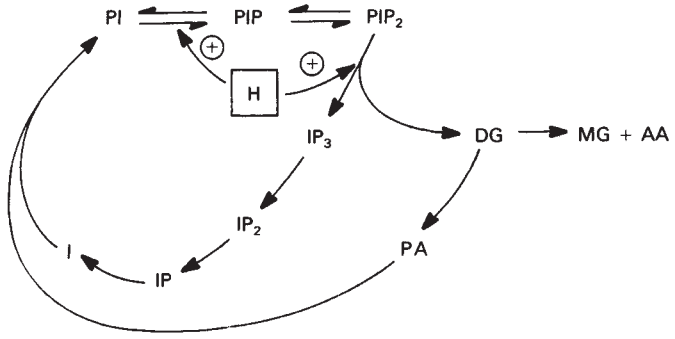

Fig. 1. *Turnover of phosphatidylinositols.* When a hormone (H) (or other extracellular messenger activates this system, it appears to produce two effects: *(1)* a rapid phosphodiesteric cleavage of phosphatidylinositol 4,5 bisphosphate (PIP_2) into a water-soluble product inositol 1,4,5 trisphosphate (IP_3), and a lipid soluble product, diacylglycerol (DG) which is rich in arachidonic acid; and *(2)* a stimulation of the synthesis of PIP_2 from phosphatidylinositol (PI) via the intermediate, phosphatidylinositol 4-phosphate (PIP). IP_3 undergoes rapid catabolism successively to inositol 1,4 bisphosphate (IP_2), inositol 1 phosphate (IP), and eventually free inositol (I). The DG can either be phosphorylated to phosphatidic acid (PA) which together with free I serve as precursors of the resynthesis of PI, or it can be acted upon by diglyceride lipase to yield monoglyceride and arachidonic acid (AA). The latter can undergo further metabolism to prostaglandins, leukotrienes, and/or thromboxanes, which is not considered in the present article.

Both IP_3 and DG serve messenger functions. The IP_3 is a water soluble messenger which enters the cytosol and induces an increase in the rate of calcium efflux from the endoplasmic reticulum [18–21]. It (the IP_3) is rapidly hydrolyzed successively to inositol 1,4 bisphosphate (IP_2), inositol 1 phosphate (IP) and eventually to free inositol (I) which then serves as a substrate for the resynthesis of PI. It is presumed that in a cell displaying a sustained response to the sustained presence of an extracellular messenger, there is a continued high rate of PI turnover, a sustained elevation of IP_3 and hence, a depletion of the endoplasmic reticular calcium pool.

In addition to a release of calcium from this pool, there is in many cells an increase in the rate of calcium influx into the cell across the plasma membrane [22–24]. The importance of this calcium influx will be discussed below. At this point it is only important to indicate that, to our knowledge, the link between the PI system and this change in plasma membrane calcium permeability is not known. It is possible that IP_3 is the messenger that regulates this process. Conversely, it is possible that a product of DG metabolism, phosphatidic acid (PA), might serve this function, or that the turnover of PIP_2 is somehow linked to the gating of calcium across this membrane.

The DG generated as a consequence of PIP_2 hydrolysis also serves a messenger function [7, 25–27]. It is thought to remain in the plasma membrane and act as a positive sensitivity modulator of a specific protein kinase, the calcium-activated, phospholipid-dependent protein kinase or C-kinase. This kinase is activated by Ca^{2+}, but this effect is not mediated by calmodulin or any known calcium binding subunit. The calcium binds directly to the enzyme. If this enzyme is extracted from non-activated cells, it is a relatively poor protein kinase and relatively insensitive to activation by Ca^{2+}. However, if it is mixed with diacylglycerol containing at least one polyunsaturated fatty acid, for example, 1-oleoyl-2-acetyl-glycerol (OAG), and a mixture of membrane phospholipids (in particular phosphatidylserine), it is transformed into a highly active kinase that is exquisitely sensitive to activation by Ca^{2+}. It is presumed that when hormone-receptor interaction leads to DG production in an intact cell, the DG binds to the enzyme, causes it to associate with phospholipids in the plasma membrane, and shift from its calcium-insensitive to its calcium sensitive form.

Discoverers of this enzyme propose that in many cellular systems the IP_3-induced increase in cytosolic free Ca^{2+} acts synergistically with the DG-dependent activation of C-kinase to determine cellular response [7, 24–27]. Our recent data [6, 8, 20, 28–30] support this view but extend it in two ways: *first*, by proposing that these two responses play distinct temporal roles in the integrated cellular response; and *second*, by emphasizing that an additional event, an increase in plasma membrane calcium influx, plays a key role in regulating a sustained cellular response [31]. Before considering these roles in detail, it is necessary to discuss recent studies dealing with the hormonal-induced changes in cytosolic-free calcium and cellular calcium metabolism. However, before closing this discussion of C-kinase, it is important to point out that this enzyme can be induced to undergo a shift from its calcium-insensitive to its calcium-sensitive form in intact cells, if these cells are treated with either OAG or with a phorbol ester such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA) [7, 32], valuable agents used to explore the role of the C-kinase in the control of cellular response.

Cytosolic calcium during cell activation

Considerable progress has also been made in our ability to measure the calcium ion concentration in the cell cytosol. Two methods are utilitarian, although neither is completely ideal for this purpose. The first is that developed by Tsien [33, 34] and uses a fluorescent derivative of EGTA, a calcium chelator, called Quin 2, which is added to a cell suspension in its esterified form. In this form it passes across the plasma membrane into the cell where nonspecific esterases hydrolyze the ester to its free acid, calcium binding form. This form displays an increase in specific fluorescence when the Ca^{2+} concentration increases. The other method, developed in several laboratories [35, 36], uses a photoprotein from jellyfish, aequorin. This protein emits light when it interacts with Ca^{2+} in the micromolar and submicromolar range. Its use in small cells, such as endocrine cells or hepatocytes, requires that the cell membrane be made reversibly permeable to the protein under conditions where Ca^{2+} is excluded, and then resealed into its normal calcium-impermeable state.

Each method has its limitation; each its advantages over the other. These will not be discussed, herein. The important fact is that with the use of either of these methods, a number of different investigators have found that when a large variety of peptide and amine hormones (which had been thought to act via the calcium messenger system) are added to appropriate target cells, there is, as predicted, an increase in the Ca^{2+} concentration within the cell. However, in the majority of these cases, this increase is transient, and the Ca^{2+} concentration returns to or nearly to its original basal value even though the cell continues to display its characteristic response. So these methods confirmed, on the one hand, what had long been suspected,

that hormones do cause an increase in the Ca^{2+} concentration of the cytosol in their target cells, but, on the other hand, provided the unexpected observation that such an increase is not sustained. This unexpected observation led naturally to a new question. The question is how does a transient message attract a sustained cellular response. Before answering this question, it is necessary to consider recent data concerning the effects of hormones on cellular calcium metabolism.

Cellular calcium metabolism

Two well known studies of the effects of extracellular messengers on cellular calcium metabolism are those performed by Borle [23], Borle and Uchikawa [37] and Schulz [22]. In the former, the effect of parathyroid hormone on the kinetics of calcium exchange in a kidney-derived tissue culture cell line was determined. In the latter, the effects of carbacholamine on calcium exchange in the acinar cells of the mammalian pancreas were analyzed. Although the methods used were somewhat different, the results obtained were similar in many respects. In both cases there was an agonist-induced increase in calcium exchange, and a slow but progressive increase in the total cell calcium. In the case of the pancreatic cells, an analysis of the time course of events revealed that within minutes of agonist addition, there is a rapid net movement of calcium out of the cell, followed by a very gradual increase back to and eventually above the value seen in control cells. At any point along this second phase of the response, addition of atropine (an antagonist of carbacholamine action) leads to a very rapid increase in total cell calcium, and then a very gradual decline of this value to that seen in control (untreated) cells. Based on recent evidence, as well as considerable earlier data, a logical interpretation of these results is that upon addition of agonist, a mobilization of calcium from the endoplasmic reticulum occurs as a consequence of an increase in IP_3 concentration. This mobilized calcium causes a rise in the Ca^{2+} concentration in the cell cytosol leading to the activation of calmodulin-dependent enzymes. One of these enzymes is the calcium pump in the plasma membrane. As a consequence of its activation, there is an increase in calcium efflux sufficient to cause a net efflux of calcium from the cell. This phase lasts a few minutes and then as the endoplasmic reticulum becomes depleted of calcium, the rate of efflux falls, and the cell begins to gain calcium slowly. The reason for this gain can only be an imbalance between influx and efflux (influx > efflux), but whether this is due to a sustained increase in influx or a decrease (after the initial increase) in rate of efflux is not known. Likewise, the events occurring after the addition of atropine can be explained. Upon addition of the antagonist, IP_3 production ceases, the endoplasmic reticulum refills largely from the uptake of calcium by the cell. At this point, the cell contains considerably more calcium than normal. This extra calcium is thought to be located largely in the mitochondria from which it is slowly released until the cell returns to calcium balance.

Studies in hepatocytes by a number of different investigators have provided evidence for a similar pattern of change in cellular calcium metabolism after phenylephrine or angiotensin II [38–44]. However, recent studies performed by Mauger et al [45] in this system have added one important new element. When either of these hormones is added to isolated hepatocytes, there is an immediate and sustained increase in the rate of calcium influx into the cell.

In our studies of hormone action in isolated adrenal glomerulosa cells, we have carried this type of analysis even further by measuring the time course of change in calcium influx, cytosolic-free calcium, and total cell calcium after angiotensin II addition [31]. Equally important, we have used physiological concentrations of angiotensin II. The addition of angiotensin II leads to an immediate (0 to 3 min) mobilization of calcium from the endoplasmic reticulum, a transient rise in the intracellular Ca^{2+} concentration (lasting 3 to 4 min), an immediate (1 min) and sustained increase in the rate of calcium influx, and an initial fall in total cell calcium within the first 5 min, a slight increase to a value approximately 80% of the control value which is then sustained for at least 40 min. Thus, one can recognize two phases of this response. An initial phase dominated by the IP_3-induced release of Ca^{2+} from the endoplasmic reticulum leading to a transient rise in the Ca^{2+} concentration in the cell cytosol and a net efflux of calcium out of the cell, and a second phase in which plasma membrane calcium influx remains high and constant, cytosolic-free calcium is close to its basal value and is constant, and total cell calcium is also constant. Given the fact that influx is high and total cell calcium constant, it is necessary to conclude that plasma membrane calcium efflux is also high, and that efflux balances influx, that is, in the activated cell during the sustained phase of its response, there is a higher than normal rate of calcium cycling across its plasma membrane (Fig. 2).

Gain control

Having reviewed hormonal effects (or other extracellular messengers) on PI turnover, cytosolic Ca^{2+} concentration, and cellular calcium metabolism, we now can consider the question of how an apparently transient rise in cytosolic free Ca^{2+} can lead to a sustained cellular response. This question can be answered in two parts: *First*, we consider the temporal roles of two branches in the calcium messenger system, and *second*, the link between these events and the hormonally-induced change in cellular calcium metabolism.

One can bypass the receptor-mediated events and cause either a transient increase in cytosolic Ca^{2+} concentration and/or an activation of the C-kinase. Application of divalent cation ionophores such as A23187 or ionomycin can be used to accomplish the first, and modulators of C-kinase activity, such as OAG or TPA, the second.

With such an approach Kaibuchi et al [27], to our knowledge, were the first to show that in the case of serotonin release from human blood platelets, A23187 and TPA acted synergistically in mediating the response. Ionophore alone caused a definite increase in cytosolic calcium concentration, the phosphorylation of the protein myosin light chain—a substrate for the calmodulin-modulated enzyme myosin light chain kinase, but a submaximal secretory response. The addition of OAG or TPA alone also led to a submaximal secretory response; there was no rise in Ca^{2+} concentration, no phosphorylation of myosin light chain, but the phosphorylation of a specific 40K protein occurred. Combined treatment with A23187 and TPA led to a maximal secretory response and the phosphorylation of both proteins. This combination of drugs mimicked the effects of a

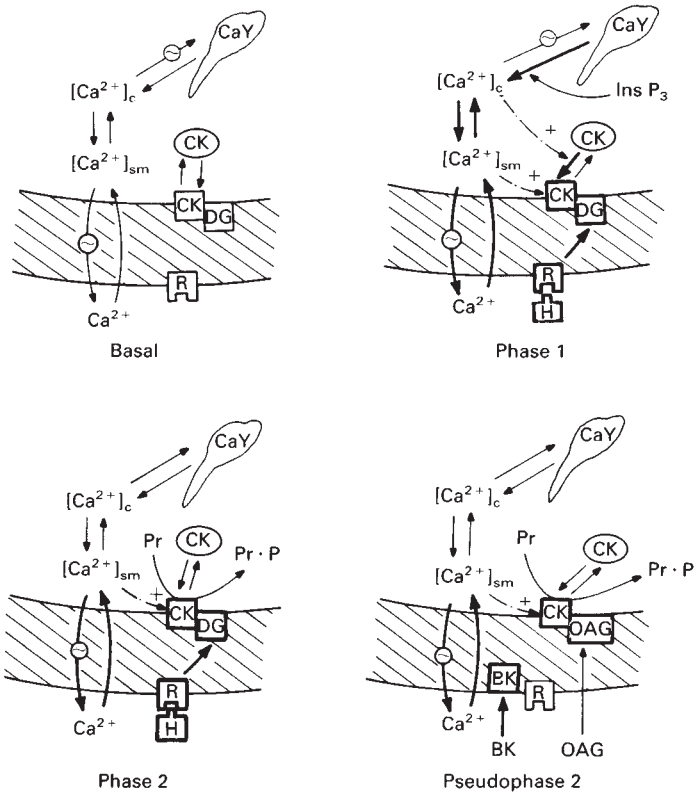

Fig. 2. *Characteristics of the two phases of cellular response when a cell is activated by an appropriate extracellular messenger which controls this response via calcium messenger system.* In its basal state (*upper left*) the cytosolic Ca^{2+} concentration $[Ca^{2+}]_c$ is low (0.1 to 0.3 μM), there is a significant store of calcium in the endoplasmic reticulum (CaY), and a low but balanced rate of calcium cycling across the plasma membrane (*shaded area*—lower portion of figure). The hormone receptor (R) is unoccupied, there is little C-kinase in its phospholipid-bound form CK; most of it exists in its nonactivated calcium-insensitive form CK. There is little diacylglycerol (DG) in the membrane. During phase 1 (*upper right*) occurring immediately after hormone-receptor interaction has initiated a response, there is a rise in the inositol trisphosphate concentration in the cell cytosol causing a release of calcium into the cytosol and a rise in the Ca^{2+} concentration in this compartment. This rise in $[Ca^{2+}]_c$ leads to the activation of the plasma membrane calcium pump and the net efflux of Ca^{2+} out of the cell. The rise in $[Ca^{2+}]_c$ serves along with the receptor-mediated increase in the DG content of the plasma membrane to induce the C-kinase to shift from its nonactivated form to its activated, phospholipid-associated, calcium-sensitive form on the endoplasmic face of the plasma membrane. Hormone-receptor interaction also leads to a prompt increase in the rate of calcium influx into the cell across the plasma membrane. Within a few minutes, the $[Ca^{2+}]_c$ regresses to or nearly to its basal value and the cell enters phase 2 of its response. In this phase the endoplasmic reticulum is depleted of calcium, the $[Ca^{2+}]_c$ is near its original basal value, but the activated C-kinase remains associated with the plasma membrane. Also, there is a sustained high rate of Ca^{2+} cycling across this membrane. This high rate of cycling causes the elevation of the Ca^{2+} concentration in a restricted, submembrane domain, $[Ca^{2+}]_{sm}$, at the endoplasmic face of the plasma membrane. The concentration of Ca^{2+} in this domain controls the rate of expression of the activated C-kinase enzymes bound to this face of the membrane. One can create a pseudophase 2 response without a prior phase 1 occurring by treating the cell with a combination of a calcium channel agonist, BAY K 8644 (BK), and an exogenous diacylglycerol, (OAG).

natural agonist, thrombin. These observations led to the conclusion that there are two pathways of information transfer in the calcium messenger system and that they act synergistically in mediating the cellular response to appropriate agonist.

Our own work has extended this concept by proposing that in a cell which displays a sustained response to the sustained presence of an agonist, the two branches of the calcium messenger system play distinct temporal roles [6, 8]. It is our proposal that the calmodulin branch, which is transiently activated by the transient increase in cytosolic-free calcium, is responsible for initiating the response; and the C-kinase branch, which is activated by the rise in DG content of the plasma membrane, is responsible for sustaining the response.

This postulate is based on data obtained from studies on insulin secretion, aldosterone secretion, prolactin secretion, and smooth muscle contraction. Although the general results and conclusions are similar from all of these systems, the most extensive analysis has been made in the case of the hormonal and ionic control of aldosterone secretion from adrenal glomerulosa cells. In these cells, there is an immediate decrease in their PIP_2 content upon the addition of angiotensin II [20]. This is associated with: a prompt and sustained increase in the contents of IP_3 and DG, an IP_3-dependent mobilization of calcium from a dantrolene-sensitive pool (presumed to be located in the endoplasmic reticulum), a transient rise in cytosolic-free Ca^{2+} concentration (unpublished observations, Apfeldorf, Rasmussen), a prompt and sustained increase in calcium influx [31], a rapid decrease of total cell calcium which is followed by a slight recovery and the maintenance of a new steady-state, and a monotonic increase in the rate of aldosterone secretion to a sustained plateau. The addition of low concentrations of A23187 do not alter PI metabolism but do cause a transient rise in the cytosolic Ca^{2+} concentration, the mobilization of calcium from an intracellular pool due to a direct effect on the endoplasmic reticulum, and a sustained high rate of calcium influx [31]. Despite the fact that A23187 induces a larger increase in cytosolic Ca^{2+} concentration and calcium influx than does angiotensin II, the aldosterone secretory response is transient. The initial phase of these respective secretory responses are quite similar, but after the response to A23187 has reached 75 to 85% of the maximal response seen after angiotensin II, it peaks and then declines progressively to values only slightly greater than its basal rate. In contrast, the addition of OAG leads to no immediate changes in PI metabolism, nor cellular calcium metabolism, and a delayed, sustained but submaximal secretory response. Combined addition of A23187 and OAG lead to an aldosterone secretory response which is temporarily and quantitatively similar to that seen after angiotensin II addition.

The simplest view of these data is that the transient calcium spike activates calmodulin-dependent enzymes which are responsible for initiating the response, but cannot, by themselves bring about a sustained response; and that the modulation of the C-kinase from its non-activated, calcium-insensitive form to its activated, calcium-sensitive form, either by exogenous or endogenous DG, complements the initiating events and is responsible for sustaining the response. In this view, once the cell is activated the system does not require messenger calcium. However, this view is incorrect, the control of this system is much more elegant than had been supposed previously. In particular, two additional aspects of its behavior are notewothy: The first concerns the role that the transient rise in the Ca^{2+}

concentration plays in causing the activation of the C-kinase, and the second concerns the role that the sustained high rate of calcium cycling plays during the sustained phase of the cellular response (Fig. 2).

As noted above, induction of a transient rise in the Ca^{2+} concentration in the cytosol is responsible for the transient activation of calmodulin-regulated enzymes. However, it has an additional function. The rise of cytosolic Ca^{2+} acts, along with the increase in DG content of the plasma membrane, to cause a shift of C-kinase from its nonactivated to its activated forms [31]. Because C-kinase molecules are not shifted from their nonactivated to their activated state after the Ca^{2+} transient, a fixed amount of C-kinase remains in its activated form throughout the sustained phase of the cellular response.

The second feature of the sustained phase is a sustained high rate of calcium cycling across the plasma membrane. This, too, plays a regulatory role. If either the extracellular K^+ concentration is reduced or a calcium channel blocker, such as nitrendipine, is added to cells displaying a sustained response the rate of aldosterone secretion promptly falls [31]. In other words the cycling of calcium plays a messenger function without a significant increase in the Ca^{2+} concentration of the bulk cytosol. However, it can only serve this messenger role in a cell in which C-kinase is in its activated form, that is, calcium cycling and C-kinase are linked as a functional unit in the plasma membrane, which controls the sustained phase of cellular response. The transient rise in cytosolic Ca^{2+} and the sustained rise in DG determine the amount of C-kinase converted to its activated, calcium-sensitive form, and the rate of calcium cycling determines the rate at which this activated enzyme expresses its function (Fig. 2). How this enzyme reads this message is not yet known, but the simplest postulate is that the rate of Ca^{2+} cycling determines the concentration of free Ca^{2+} in a very restricted cellular domain at or within the endoplasmic face of the plasma membrane, and that the membrane-associated C-kinase directly responds to this elevated Ca^{2+} concentration rather than to this enhanced rate of cycling.

Noteworthy is the fact that one can bypass the phase 1 (initial) aldosterone secretory response and create a pseudophase 2 (sustained) response by treating the cells with a combination of a calcium channel agonist, BAY K 8644, and an activator of C-kinase, OAG [31]. Addition of BAY K 8644 alone causes an increase in rate of calcium cycling across the plasma membrane but does not cause a significant rise in the cytosolic Ca^{2+} concentration so it alone does not induce a phase 1 aldosterone secretory response. Nonetheless, when combined with OAG, a prompt and sustained secretory response is observed which is considerably more rapid in onset and displays a higher sustained plateau than that sustained response induced by OAG alone, but is only 50% of the maximal response seen when a phase 1 response precedes a phase 2 response.

Of particular interest is the fact that in the same cell type, adrenal glomerulosa cells, another peptide hormone, ACTH, also uses Ca^{2+} as a messenger and induces a sustained cellular response, but does so without activating the C-kinase branch of the calcium messenger system [46, 47]. The mechanism by which gain control is achieved during the action of ACTH is equally as elegant as that by which it is achieved during angiotensin II action (Fig. 3). Rather than using the C-kinase

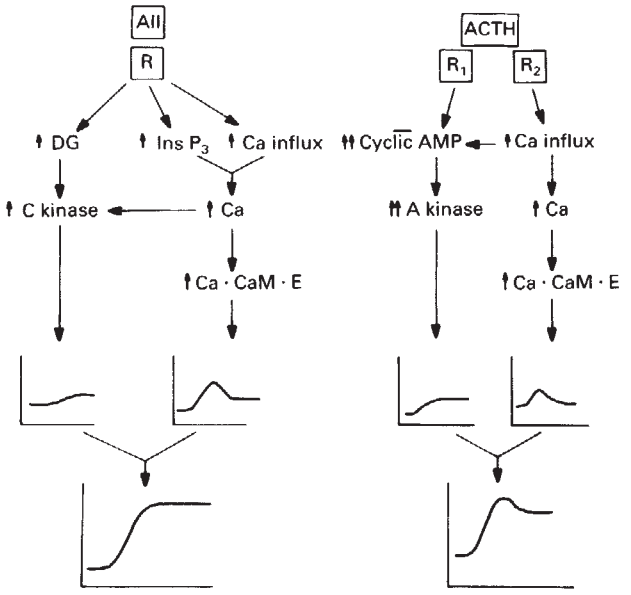

Fig. 3. *Comparison of the flow of information when angiotensin II* (left) *and ACTH* (right) *act on adrenal glomerulosa cells to induce a sustained cellular response.* When AII acts, information flows through two (or three) branches of the system. Hormone-receptor interaction leads to the generation of diacylglycerol (DG), inositol trisphosphate (InP_3), and an increase in plasma membrane calcium influx. The rise in IP_3 leads to the mobilization of Ca^{2+} from the endoplasmic reticulum which leads to a rise in the Ca^{2+} concentration in the cytosol. This rise in Ca^{2+} concentration initiates the activation of calmodulin-regulated enzymes (CaM · E). Because the rise in Ca^{2+} concentration is transient, the activation of the CaM · E is also transient, and only a transient aldosterone secretory response is induced by the flow of information through this branch. The rise in the DG content of the plasma membrane along with the transient increase in the Ca^{2+} concentration of the cell cytosol induces a shift of C-kinase from its nonactivated to its activated form. Regulation of the expression of this enzyme depends on plasma membrane Ca^{2+} cycling (not shown). If only this pathway is activated, a slowly developing and submaximal response is seen. However, when the flow of information via both branches is integrated, temporally, a maximal, sustained secretory response is observed. In the case of ACTH two surface receptors are involved, R_1 and R_2. Binding of ACTH to R_2 increases plasma membrane calcium influx which causes a transient increase in the cytosolic Ca^{2+} concentration, the activation of calmodulin-dependent enzymes, and a transient secretory response. Binding of ACTH to R_1 leads to an activation of adenylate cyclase, a rise in cyclic AMP content, the activation of cyclic AMP-dependent protein kinase (A-kinase), and a sustained but submaximal secretory response. If both receptors are activated simultaneously, information flows via both branches and an additional effect is seen; the increase in calcium influx induced by ACTH binding to R_2 acts to enhance the effect of the ACTH (bound to R_1) on adenylate cyclase activation. Also not depicted is the increased activity of the cyclic AMP-dependent protein kinase which, in turn, increases the sensitivity of CaM · E to activation by calcium. When information flows simultaneously via both branches, an integrated cellular response is seen.

control device, the cell activates the cyclic AMP messenger system to modulate events in the calcium messenger system. In acting on these cells, ACTH works via two classes of surface receptors. One class, a high affinity but low capacity one, is linked to calcium gating in the plasma membrane; the other class, a lower affinity, but high capacity class, is linked to adenylate cyclase. The intracellular events occurring as a consequence of ACTH-receptor interactions are linked in two ways. The influx of Ca^{2+} resulting from the binding of ACTH to

its first class of receptors, sensitizes the adenylate cyclase system to activation by ACTH bound to its second class of receptors. Hence, Ca^{2+} influx, a process controlled by the high affinity class of receptors, acts as a positive feedforward modulator of the activation of adenylate cyclase, a process controlled by the lower affinity class of receptors. The second type of interaction between Ca^{2+} and cyclic AMP is at the level of calcium messenger function. A rise in cyclic AMP concentration, acting via cyclic AMP-dependent protein kinase, leads to the phosphorylation of one or more calcium-regulated, calmodulin-dependent response elements, and alters in a positive way, the sensitivity of these response elements to activation by Ca^{2+}. The cyclic AMP-dependent phosphorylation of these response elements makes them more sensitive to activation by Ca^{2+}, that is, they are turned on by lower concentrations of Ca^{2+}, concentrations similar to those found normally in the cell cytosol. Hence, cellular response is sustained in the face of a transient increase in the Ca^{2+} concentration of the cell cytosol because of the positive sensitivity modulation of calmodulin-dependent response elements by cyclic AMP-dependent protein kinase (a detailed discussion of the concept and importance of sensitivity modulation has been presented elsewhere [4]). In these cells the action of ACTH involves the synarchic messengers, Ca^{2+} and cyclic AMP, interacting in both hierarchical and sequential patterns to achieve an integrated cellular response.

Although these two different mechanisms can operate independently of one another, they can also act synergistically in the sense that in the adrenal cells, as well as a variety of other cells, a rise in the cyclic AMP concentration enhances the response induced by events in the C-kinase branch of the calcium messenger system. Exactly how cyclic AMP influences events in this C-kinase branch is not known, but it is already clear that its action involves effects other than, or in addition to, effects on cellular calcium fluxes and concentrations.

Action of renal hormone. Since the present series of papers focuses on renal cell metabolism rather than transport, our major discussion of the action of renal hormones will be confined to a consideration of the actions of PTH and angiotensin II on the process of gluconeogenesis in the cells of proximal renal tubules. There are a number of recent reviews which summarize earlier work in this field [9, 10], and discuss its relevance to the effects of these hormones on various transport processes [10–12]. In addition, the possible roles of cyclic AMP and Ca^{2+} in the action of vasopressin in transporting epithelial has been discussed throughly recently [11].

Angiotensin II and renal gluconeogenesis. The available data argue that this hormone acts to control renal gluconeogensies in much the same way it acts to regulate hepatic glucose production and aldosterone secretion in adrenal glomerulosa cells. Hormone addition to isolated renal tubules leads to a hydrolysis of PIP_2 [48], a calcium-dependent increase in glucose formation [49] without a rise, but in fact a fall [50], in the cyclic AMP content of the tubules. There are no published data which evaluate the respective roles of the calmodulin and C-kinase branches of the calcium messenger system in angiotensin II-mediated gluconeogenesis, but it is reasonable to predict that each branch will play a different temporal role in regulating this tissue response.

PTH and renal gluconeogenesis

Considerably more information is available concerning the effects of PTH on renal gluconeogenesis. In this case, it is quite clear that cyclic AMP plays an important messenger function [50–53]. Likewise, there is considerable data showing that Ca^{2+} is involved [53, 54], although opinion is divided as to whether or not Ca^{2+} serves a classic messenger role in PTH action. There are at least two possible models either of which could account for the available data. Either Ca^{2+} is a true messenger in the sense described for Ca^{2+} in the actions of angiotensin II and ACTH on adrenal glomerulosa cells or Ca^{2+} is required for the expression of cyclic AMP action. In such a model system one of the effects of cyclic AMP would be to act as a positive sensitivity modulator of calcium-regulated enzymes. In this regard, it is interesting that the stimulatory effect of exogenous cyclic AMP on glucose formation from certain substrates, that is, citrate, α-ketoglutarate, succinate, and malate depends on calcium.

Most interesting data concerning PTH action are those showing that low concentrations of Ca^{2+} may be necessary for or enhance the activation of the adenylate cyclase by PTH: a situation reminiscent of that described for ACTH action in the adrenal cortex [55–57]. For this analogy to hold, one would have to postulate that there are two classes of PTH receptors on the renal tubular cell: a high affinity, low capacity class linked to Ca^{2+} entry, and a lower affinity high capacity class linked to the cyclase. The general belief is that only a single class of PTH receptors exists, but this opinion is not unanimous. Parenthetically, a similar uncertainty existed for a long time in the case of ACTH receptors on adrenal cells. Further analogies exist between ACTH action in glomerulosa cells and PTH action on renal cells. In both cases many of the effects of exogenous cyclic AMP depend on the presence of Ca^{2+}. On the other hand, there are reports that PTH stimulates PI turnover in renal cells, an action not produced by ACTH. However, the studies done to date with PTH address only the issue of PI synthesis [48, 58–60]. No one, to our knowledge, has shown that PTH stimulates the hydrolysis of PIP_2 and the production of IP_3 and DG. Until such experiments are performed, the question of whether or not PTH mediates some of its effects via the C-kinase branch of the calcium messenger system remains an open one.

Parenthetically, it would be of considerable general interest if the same peptide hormone brought about the activation of both the cyclic AMP messenger system and the complete calcium messenger system, as do catecholamines acting on the liver, that is, the calcium messenger system is linked to the alpha receptor and the cyclic AMP messenger system to beta receptor. If PTH were found to activate the calcium messenger system, this would be very strong indirect evidence for the existence of two classes of PTH receptors. In considering this possibility, it should also be made explicit that when ACTH binds to its high affinity class of receptors, it brings about an increase in the rate of plasma membrane calcium influx without activating PIP_2 hydrolysis. In this case, the only recognized change in cellular calcium metabolism is a sustained increase in the rate of calcium cycling across the plasma membrane. In contrast, when catecholamines act in the liver cell, activation of

alpha receptors leads to PIP_2 hydrolysis and a similar sequence of events as those seen in the case of angiotensin II action. Thus, certain hormone receptors can activate the full calcium messenger system, and others only a single component of it. The molecular basis for this selective activation of a single component is not yet known. The relevance of this discussion to the mechanism of action of PTH on the renal tubule is that either model of PTH action is possible based on the available data. The experimental methods are now available with which to test these alternative models in renal cells. From such experiments, it should be possible to define which model more adequately accounts for the control of renal gluconeogenesis by PTH.

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